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Project Completion Report

TO DEVELOP A SHORT AND LONG TERM PROGRAM AIMED AT THE CONTROL AND ELIMINATION OF TCK SMUT

Prepared for

Old West Regional Commission
The Fratt Building, Suite 306 A
Billings, Montana, 59101

Submitted by

Robert Brastrup, Executive Secretary
Montana Wheat Research and Marketing Committee
The First National Bank Building, Room 310
Great Falls, Montana, 59401

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INTRODUCTION

The Peoples Republic of China (PRC), early in the year of 1974, stated that they would no longer purchase wheat from Pacific Northwest ports. They claimed that their country was free of TCK (Tilletia controversa Kuhn) smut. The PRC subsequently placed a zero TCK tolerance on all wheat entering their country and would not purchase wheat from areas known to be contaminated.

Prior to the elimination of the Pacific Northwest as a supplier of wheat, the PRC had purchased approximately 30.5 million bushels that were loaded out of the Pacific Northwest ports during the 1973-74 marketing year. By the end of that marketing year, they had purchased approximately 118 million bushels from the U.S. as a whole. The PRC had been a significant market for wheat produced in the Pacific Northwest area.

In an attempt to regain this market, the Pacific Northwest States' Wheat Commissions, Departments of Agriculture, and the Grain Trade drew up a research proposal composed of seven tasks. The first year of the project proposal was funded by the Old West Regional Commission. The Grant had a beginning date of December 27, 1974. Due to the fact that not all research tasks, under this grant, were able to begin at the same time, the later tasks were allowed an extension for completion of their contracts. This report describes the work done during the first year.

The complaint by the PRC was the first of its kind involving TCK smut which was, heretofore, thought to be, solely, a domestic

problem. Thus, in the past, there had been no extensive attention directed toward TCK research.

With regard to the PRC, it was believed that TCK could develop as a problem for all U.S. ports because: 1) many other winter wheat growing areas may have TCK infestation and not be aware of it, 2) TCK spores in grain could be transported to all parts of the U.S. and be mixed with and contaminate normally clean grain, 3) the transporation vehicles themselves could retain TCK spores from previous shipments of contaminated grain.

The project was approached realistically in that those involved realized that this multi-faceted problem could not be conquered in one year. However, this first year of work has laid an important base for the succeeding years of the project.

TASK 1

DEVELOPMENT OF PROCEDURES FOR THE DETERMINATION OF VIABILITY OF TELIOSPORES OF TILLETIA CONTROVERSA (TCK)

Supported by

The Old West Regional Commission
and
The Montana Wheat Research and Marketing Committee

Submitted by

J. A. Hoffmann, Research Plant Pathologist

Marianne Anderson, Research Assistant

ARS-USDA, Crops Research Laboratory

Utah State University, Logan, Utah

Description of the Problem

The opening of trade with the People's Republic of China (PRC) provides a new and potentially large market for United States wheat. Initial efforts to export western wheat to the PRC were disrupted because the grain was often contaminated with spores of Tilletia controversa Kühn, (TCK), the fungus causing the dwarf bunt disease of wheat. The Chinese claim that dwarf bunt is not present in their country and are concerned that its introduction may pose a threat to their own wheat production. They have, therefore, established a quarantine prohibiting the import of wheat containing spores of T. controversa, which, in effect, excludes western wheat from the PRC market.

Reentry of western wheat in the PRC market will require a solution to the TCK contamination problem. One immediate solution would be the development of commerically applicable procedures to remove or kill the dwarf bunt spores contaminating the grain. However, regardless of what procedures might be developed for sanitizing grain, concommitant procedures will be needed for determining that the spores are no longer viable. Such a technique will be useful to the researcher in developing sanitization treatments as well as to the grain traders (US & PRC) to insure that the treatment is effective.

At present, the only way to determine the viability of dwarf bunt spores is to attempt to germinate them. Germination of dwarf bunt spores requires cold temperatures (optimum 5C), light, and an incubation period of from 3-8 weeks. Thus, germination tests are laborious and time consuming at best and may be totally impractical with the small number of spores recovered from grain samples.

The use of tetrazolium salts as indicators of viability has become common

practice with seeds and other plant organs. Other chemical and physical techniques for assessing viability have been reported recently. It seemed possible that one or more of the techniques used for determining viability in other organisms might be developed into a quick and reliable procedure for determining viability of dwarf bunt spores.

Objective

To develop a quick and reliable procedure for determining the viability of dwarf bunt spores. The test should be relatively simple, sensitive, and precise, and should clearly differentiate viable from nonviable spores.

Materials

Dwarf bunt (Tilletia controversa) teliospores were obtained from smutted wheat heads produced at the Blue Creek Experiment Station, Box Elder County, Utah in 1973. Spores of common bunt, Tilletia foetida (race L-8) and T. caries (race T-16), were also used in some tests.

Spore viability was determined by germination tests on soil extract agar. For dwarf bunt, germination plates were incubated at 5C under continuous, low-intensity, fluorescent light. Common bunt spores were germinated at 15C in either light or dark. All spore collections used exhibited germinations percentages exceeding 90%. In tests using dead spores, the spores were killed by autoclaveing them for 20 minutes or by soaking them in 50% ethyl alcohol for 30 minutes.

Methods and Results

I. Peroxidase

The peroxidase method has been used as an indicator of pollen viability (J. R. King). A differential color reaction in living and dead cells is based on oxidation of benzedine by peroxidase in the presence of hydrogen peroxide. Viable cells are stained dark blue whereas nonviable cells remain unstained.

Experiments using this technique were conducted with T. controversa and T. foetida. No reaction was obtained with spores of either species on agar or in suspension in vials protected from light at several different temperatures.

II. Triphenyl tetrazolium chloride

2-3-5-triphenyl tetrazolium chloride has been used successfully as an indicator of seed viability, germinability of dormant seeds, and viability of plant tissues (Baird, Lakom, Muentz, Overaa, Smith, etc.).

Triphenyl tetrazolium chloride is a colorless solution which is reduced to the stable and nondiffusable red formazan by living cells. Necrotic cells remained unstained. The reaction is based upon the reduction of dehydrogenase, a respiratory enzyme with high activity in germinating seeds.

Spores of T. foetida and T. controversa on agar plates were exposed to various concentrations of 2-3-5-triphenyl tetrazolium chloride in dark chambers at 5 and 25C. In addition, some spores were subjected to various pretreatments (cracking the spore wall by shaking with seeds, soaking in 5% clorox, soaking in 10% DMSO) prior to plating and exposure to the stain. In all cases, neither the viable nor dead spores reacted to the stain.

In a separate experiment, spores of both species were mixed with solutions of triphenyl tetrazolium chloride in vials wrapped in aluminum foil. Stain concentrations used were 0.5, 1, 2, 3, 5, and 7%; the pH of the stain solutions was 6-7; The experiment was conducted at room temperature (20C).

Approximately 10-20% of the viable spores of T. foetida were stained light red within 5-8 days, whereas dead spores remained almost colorless. However, no staining of either viable or dead spores occurred with T. controversa.

The failure of the preceding experiments with T. controversa were suspected as being due to the highly resistant and impermeable spore wall. In attempts to increase the permeability of the spore wall, experiments were conducted in which

the spores were subjected to the following pretreatments:

1. Pregermination: Spores of T. controversa were kept on agar in the refrigerator at 5C for different periods of time up to three weeks and then mixed with 2-3-5-triphenyl tetrazolium chloride. Only spores which were pregerminated for at least 2½ weeks showed a slight staining reaction.

2. Soaking in sucrose solutions: Sucrose has been reported to be an activator for enzymes such as dehydrogenase which are involved in glucose metabolism. The spores were soaked for 20-48 hours in 0.4M, 0.5M, 0.6M, 0.8M, 1.0M, and 2.0M sucrose solutions at room temperature and then mixed with 2-3-4-triphenyl tetrazolium chloride of different concentrations. No improvement of the stain reaction in viable spores was detected.

3. Soaking in water: Soaking the spores in water for 48 hours in a dark chamber at 25C or 5C prior to staining did not improve the stain reaction.

4. Soaking in pectinase: T. controversa spores have a wall consisting of several distinct layers. The outer wall is apparently composed of various pectin compounds and may be affected by pectinase. In an attempt to increase permeable, spores were suspended in a pectinase solution for 28 hours in vials kept in the dark at room temperature. After 28 hours the spores were rinsed with distilled water, suspended in tetrazolium solution, and kept in a dark chamber at room temperature.

Viable spores of T. controversa showed some color reaction with the staining solution within 3 days, whereas dead spores remained colorless. The best staining reaction (about 35% of viable spores stained) occurred when spores were soaked in pectinase at pH 5.3, rinsed with distilled water, and mixed with 1% tetrazolium solution in pH 7 phosphate buffer. This procedure was combined with pregermination of spores of agar for 7-15 days, but there was no noticeable improvement in the percentages of vaible spores

stained.

5. Soaking in cellulase and protease- At the suggestion of Dr. E. J. Trione, spore of T. controversa were treated with the enzymes cellulase and protease. The spores were suspended for 2, 3, 4, 5, and 7 hours in buffered cellulase and protease in vials wrapped in aluminum foil and shaken in a water bath at 37, 47, and 50C. Although the enzyme treatments did not improve the staining reactions, it is of interest to note that spores of T. controversa were still viable following the enzyme treatments. This was demonstrated by germinating the spores on agar.

III. Other Tetrazolium Compounds

All the previously described methods were conducted also with the following tetrazolium compounds:

1. Neotetrazolium chloride: This compound, which is supposed to stain viable cells red, showed no consistent reaction.

2. Tetrazolium blue: This compound did not show any staining reaction at concentrations of 0.5, 1, or 5%.

3. Thiocarbamyl nitro blue: This compound produced a staining reaction after pretreatment of spores in cellulase and protease. Viable spores suspended in cellulase and protease for 4.5 hours in a water bath at 48C were stained brown to black within 1½-2 days. In one experiment 80% of the viable spores stained dark red, whereas 50% of the dead spores were stained. In this experiment the spores were soaked for 4.5 hours in cellulase and for 3.5 hours in protease while gently shaken at 38C. The spores were then suspended in 1% thiocarbamyl with phosphate buffer (pH 7). The stain appeared within 22 hours and did not change during the following 48 hours. However, all repetitions of this experiment, and others, with

different concentration (2%, 3%, 5%) of thiocarbamyl, did not confirm the previous result.

The best differential staining was obtained by pretreatment of spores for 4 hours in cellulase and 4.5 hours in protease at 50C followed by a carbamyl stain concentration of 2% with phosphate buffer (pH 6.5). About 45% of the viable spores were stained purple, whereas the dead spores remained colorless. This experiment was repeated three times with fairly consistent results. However, no increase in the percentage of viable spores stained was achieved.

4. MTT tetrazolium: This compound showed the most active staining of all the tetrazolium compounds tested. It was the only one which showed staining activity, even without the cellulase/protease pretreatment. Experiments were conducted with pretreatments such as soaking in water for 44 hours, soaking in sucrose (0.4M) for 20 hours, pregerminating on agar for 2.5 weeks, and soaking in pectinase (pH 5.3). The spores were then treated with MTT solutions of 0.5, 1, 2, 3, or 5% concentration. Results were somewhat improved by adding 1ml DMSO to 10ml of stain solution. However, the dead spores showed about the same reaction. Pretreatment of spores in pectinase also produced some improvement in stain uptake but did not differentiate viable from nonviable spores to a sufficient degree. Spores soaked in cellulase for 3.5 hours and in protease for 4.5 hours at 38C, and then mixed with 1% MTT with a phosphate buffer (pH 6.5) showed the following results:

30% viable spores stained - 20% dead spores stained after 17 hours

70% " " " - 20% " " " " 23 hours

95% " " " - 95% " " " " 49 hours

There was no indication that percentages of MTT higher than 1% improved

staining activity, whereas 0.5% MTT seemed to be too weak. Some spores stained by MTT were purple, whereas most were black.

IV. Post-Treatments

Referring to reports that formazan is soluble in sodium hyposulfite, ethanol, methanol, glacial acetic acid, and acetone, these post-treatments of stained spores were included in our experiments:

1. Sodium hyposulfite: The stained spores (viable and dead) were soaked for 16 minutes in sodium hyposulfite in a water bath at 38C, and rinsed 5 times with distilled water. Microscopic examination of the spores showed that some stain had been removed by the treatment. There appeared to be a greater differentation between the viable and dead spores after the post-treatment, but the difference was still not sufficiently distinct.

2. Ethanol: Referring to Harty's "Quantitative reduction of triphenyl tetrazolium" we attempted to extract the red formazan from the spores with 95% ethanol for 30 minutes. (Previous experiments showed that this concentration of ethanol does not kill spores of T. controversa.) The absorbance of the extract from living and dead spores was read with a spectrophotometer. There was no discernable difference between the extracts from dead and viable spores. Washing spores with 50 and 95% ethanol likewise did not show differences between viable and dead spores when observed microscopically.

3. Methanol and glacial acetic acid: Stained spores were extracted with 1:1 glacial acetic acid and methanol at room temperature for 15 hours. There was no difference between viable and dead spores examined spectrophotometrically.

4. Acetone: Spores stained with triphenyl tetrazolium and MTT, placed on a micro slide and treated with acetone showed a surprising effect. As described previously, viable and dead spores of T. controversa stained by

triphenyl tetrazolium chloride, thiocarbamyl nitro blue, and MTT were stained to almost the same amount. When acetone was added to the slide under the microscope the stain in the dead spores faded, whereas the viable spores (about 60%) remained stained red to black. Acetone evaporates very rapidly and the difference could be seen for only a few seconds. However, attempts to repeat these results were highly inconsistant.

V. Fluorescent Methods

Spores of T. controversa are somewhat fluorescent even without treatment, often producing a yellow, green, dull-red, or bright red fluorescence. There is apparently no difference in natural fluorescence between viable and dead spores.

1. Fluorescein diacetate: This method was successfully used as a test of viability by Harrison, Heslop, and Greenberg and was attempted with various modifications by us with T. controversa spores. Fluorescein diacetate was dissolved in acetone (2mg/ml) and added drop by drop to sucrose solutions (0.2M, 0.4M, 0.6M, 0.8M, 1.0M, and 2.0M) until the first persistent milkiness appeared. This approach was varied by:

(a) Spores mixed with fluorescein diacetate dissolved in acetone without sucrose.

(b) Fluorescein diacetate (0.5%, 1%, 0.75%, 2%) dissolved in water without acetone, with and without sucrose.

The various procedures used produced no apparent difference in fluorescent staining of the spores.

2. 4-Methyl-umbelliferone myristrate, Fluorescein dibutrate, and p-Nitrophenyl myristrate: These fluorescent compounds are reported to be indicators for the enzyme lipase. Because no special descriptions for the use of these compounds were available, we used essentially the same procedures as used with fluorescein diacetate. None of the materials provided a definite and

repeatable differentiation between viable and dead spores.

3. Lactophenol: In this method the spore wall of viable spores were reported (Hampson) to be fluorescent red and their contents fluorescent green, whereas dead spores were supposed to be a dull red. In adapting this method to spores of T. controversa, the spores were suspended in lactophenol for several minutes, then placed in 0.1% Triton 100 phosphate buffered saline, and mixed with various flurochrome solutions such as basic fuchsin, rose bengal, and acriflavin HCL. In all cases, dye uptake was negligable and inconsistent.

Summary and Discussion

As a result of our experience in attempting to develop methods for differentiation of viable and dead T. controversa spores, we have concluded that the impermeability of the spore wall presents the main problem in developing a suitable staining technique. Consequently, future work in this area should be concentrated on the development of pretreatments to increase the permeability of the spore wall.

Of the tetrazolium compounds tested, MTT appeared to be the one with the greatest staining activity. Post-treatment with acetone showed some initial promise but did not prove to be consistently reliable. Methods involving the use of various fluorescence-inducing materials showed no encouraging results whatsoever. The impermeability of the spore wall and the fact that the spores are naturally fluorescent appeared to present overriding complications.

Because of the lack of necessary instrumentation, methods, involving the detection and assay of adenosine triphosphate (ATP) were not investigated. This technique has been used successfully to determine viability in bacteria and spores of the fungus Synchytrium endobioticum and may be adaptable to spores of the smut fungi. Studies to determine the applicability of ATP assay methods to the smut fungi will be pursued as soon as arrangements can be made for use of an ATP photometer.

TASK 2

ESTABLISHMENT OF DWARF BUNT EXPERIMENTAL NURSERIES

Supported by

The Old West Regional Commission
and
The Montana Wheat Research and Marketing Committee

Submitted by

Vern R. Stewart, Superintendent

Montana State University

Northwestern Agricultural Research Center

Kalispell, Montana

J. A. Hoffmann, Research Plant Pathologist

ARS-USDA, Crops Research Laboratory

Utah State University, Logan, Utah

Stillwater Site - Crop Year 1974-75:

Very dry conditions existed at this location in the fall of 1974. Because of these conditions, there was no emergence of winter wheat until early spring of 1975. Stands were very erratic. Because the wheat emerged in the spring, these plants escaped infection by the dwarf bunt organism. As a result, we did not obtain any data from this location in 1974-75.

Crop Year 1975-76, Studies Conducted by Dr. J. A. Hoffmann:

Eight hundred and seventy-eight lines were included in a dwarf bunt screening study. Material for this test was supplied by wheat breeders in Montana, Idaho, Washington, Oregon, and Utah. All lines were inoculated with spores obtained from this area.

These lines were evaluated for dwarf bunt mid-August 1976. It should be noted that there was a very high level of dwarf bunt in such susceptible varieties as Wanser and Westmont. Because of the high level of dwarf smut, we feel that any line which is free of dwarf bunt is, indeed, resistant to the races of dwarf bunt found in this location.

One hundred and fifteen (115) lines were found to have 1% bunt or less in this evaluation. Agronomic data and quality data will have to be obtained for these lines in the next season.

Fifteen fungicides at various rates were tested using the very susceptible variety Wanser. Wheat seed was treated with each of the fungicides prior to seeding. Only TBZ (Mertect 360) at rates of 1, 2, and 4 oz/bu gave any degree of control. The results were: untreated check 65% smut, 1 oz rate, 2%; 2 oz, 3%; and the 4 oz rate, 2%.

Crop Year 1975-76, Studies Conducted by Vern R. Stewart:

The Western Regional Hard Red Winter and White Wheat Nurseries were grown in this location. These nurseries contained 28-30 lines each from wheat breeders in the states listed above. These were planted in a soil containing a high level of dwarf bunt inoculum. We do not inoculate artificially as Dr. Hoffmann does.

We feel the high level of bunt in the susceptible varieties this season gives us a good test on all lines in the test. We did find some lines with a degree of resistance. McDermid, the newly recommended variety for western Montana, contained up to 10% smut. Crest, also a recommended variety, contained over 10% bunt in this test. We have noted the continuing rise of bunt in Crest for the past four years.

Northwestern Agricultural Research Center site - Crop Year 1974-75:

During this crop year, we grew the Western Regional Hard Red

Winter Wheat Nursery and the Western Regional White Wheat Nursery. These nurseries contained entries from plant breeders in the western area of the United States, mainly the states of Montana, Idaho, Washington, Oregon and Utah. From these nurseries we secured agronomic yield and disease data.

Dr. Allan Taylor had over 500 lines in this location for dwarf bunt evaluation. These lines were from his breeding program which include lines that should have a potential for dwarf bunt resistance.

Elgin, a dwarf bunt susceptible variety, contained 16% bunt. This level of bunt, though not too high, did give some indication of the resistance of some lines in the test. In the Western Regional White Wheat Nursery, Moro and McDermid showed good resistance to the disease. With this year's data and some three additional years' data McDermid was added to the list of recommended varieties for western Montana, for planting in the fall of 1975.

Dr. Taylor was able to make some selections on early generation material with the bunt level found in this location in 1975.

Crop Year 1975-76:

The two regional nurseries described above (1974-75) were grown in the 1975-76 crop year. A very open winter of 1975-76 with little or no snow cover, resulted in little or no dwarf bunt in this location. Thus, we could not make any dwarf bunt evaluation on lines grown in these nurseries.

Looking Ahead:

Nine acres of land has been secured from Mr. Lance Claridge to increase research on dwarf smut in this location.

This additional acreage should allow us to look at more genetic material, increase studies on chemical control, and perhaps some work on cultural techniques. At this writing, it would appear that a combination of genetic resistance, chemical control, and cultural methods may result in the best control of dwarf bunt (TCK).

In the fall of 1976 we seeded (1) two fungicide studies, (2) two regional nurseries and (3) several hundred lines of breeding material from the western states mentioned above.

TASK 3

THE DEVELOPMENT OF TESTS TO IDENTIFY DWARF BUNT SPORES

Supported by

The Old West Regional Commission
and
The Montana Wheat Research and Marketing Committee

Submitted by

Edward J. Trione, Research Biochemist, ARS

B. Bruce Krygier, Biological Laboratory Technician

Department of Botany and Plant Pathology

Oregon State University, Corvallis, Oregon

THE DEVELOPMENT OF TESTS TO IDENTIFY DWARF BUNT SPORES

Final Report on the TCK Smut Identification Project Supported by
The Montana Wheat Research and Marketing Committee

Project Number 7503 March 1 to Dec. 31, 1975

Edward J. Trione, Research Biochemist, ARS
B. Bruce Krygier, Biological Lab. Technician
Dept. Botany and Plant Path.
Oregon State University
Corvallis, Oregon 97331

BRIEF DESCRIPTION OF THE PROBLEM

The Peoples Republic of China set a zero tolerance on dwarf bunt spores (also known as dwarf smut or TCK spores) and will reject any shipments of wheat containing spores that look like TCK spores. There is considerable similarity in the external appearance of the spores of the dwarf, common and grass smuts. The morphological overlap between these species is so great that when only a few smut spores are available for observation they cannot be positively identified by observing them with the light microscope or even with the high magnification of the scanning electron microscope. Wheat shipments containing common or grass smut spores would be accepted by the Peoples Republic of China if it could be shown that TCK spores were not present.

OBJECTIVES

The development of tests that will give a positive identification of TCK spores, when only a small sample of spores is available, i.e. 5 to

100 spores. These tests should be sensitive, rapid and precise and should clearly distinguish TCK spores from common and grass smut spores.

BRIEF DESCRIPTIONS OF SMUT SPORES

TCK Spores (dwarf smut, Tilletia controversa): Spherical, 19-24 microns in diameter. Large, regular polygonal surface reticulations, 1.5 to 3 microns deep. An almost imperceptible hyaline sheath, 1-5 microns thick, usually surrounds these spores.

Common Smut Spores (Tilletia caries): Spherical, 14-25 microns in diameter. Irregular polygonal surface reticulations, 0.5 to 3 microns deep. A hyaline sheath may or may not be present.

Grass Smut Spores (Tilletia fusca group): Spherical, 18-32 microns in diameter. Large polygonal surface reticulations, 1.5 microns deep. A hyaline sheath may or may not be present.

The average weight of these different types of smut spores is very similar.

500 spores weigh 1 microgram

100 spores weigh 0.2 micrograms

5 spores weigh 0.01 micrograms

In consideration of the desire to develop specific tests using only 5 to 100 spores it was obvious that no conventional chemical analyses of amino acids, carbohydrates, lipids, proteins, etc. would be sensitive enough for these submicrogram samples.

RESULTS

ENZYMATIC HYDROLYSIS OF THE SPORE WALL

Our first experimental approach was based on the hypothesis that there was chemical similarity in the cell walls of smut spores within one species, but that there were chemical differences in the cell walls of TCK, common and grass smut spores.

If such differences exist they may be detected by treating the various spore types with unique enzymes, known to be capable of hydrolyzing portions of fungal cell walls, and then viewing the spores at very high magnification with the scanning electron microscope. Hopefully, after a specific reaction sequence, the spore types that previously looked similar would then look quite different and permit distinction of TCK from the other smut spores.

Based on knowledge of the chemical constitution of fungal spore walls eight different enzymes were chosen, each of which was potentially capable of removing some of the cell wall material. The eight enzymes studied were:

Enzymes	Substrates hydrolyzed
Cellulase	Cellulose (β-1, 4 glucan)
Laminaranase	β-1, 3 glucans
Amyloglucosidase	α-1, 4 and α-1, 6 glucans
Chitinase	Chitins
Protease	Proteins

Enzymes	Substrates hydrolyzed
Hemicellulase	Polysaccharides (non-cellulose)
Lysozyme	Muramic acid polymers
Pectinase	Pectins

The reaction mixtures contained 50 mg spores and 25 mg enzyme in 50 ml of buffer. Protease and lysozyme reactions were buffered at pH 7.0 with 0.1 M phosphate and all other enzyme reactions were buffered at pH 5.0 with 0.1 M sodium acetate. All enzyme reactions that lasted longer than 4 hours contained sodium ethylmercurithiosalicylate (thimersol) at 0.25 millimolar concentration to prevent microbial contamination.

In addition to treating the smut spores with the individual enzymes, various combinations of enzymes were tested for their combined effect. After 2 to 4 hours in the reaction mixture the spores were filtered from the suspension, air-dried in a vacuum oven and viewed with a scanning electron microscope, usually at 1000 to 10,000 x magnification. No quantitative measurements were made on the degree or extent of spore wall hydrolysis. Visual comparisons were made on the degree or extent of spore wall hydrolysis, and the relative effects of these enzyme treatments on TCK and common smut spores were noted. Many of the enzyme treatments appeared to have partially degraded the spore wall, as seen in Figure 1, but no enzyme treatment was found which enabled us to clearly distinguish TCK from common smut spores. Grass smut spores were not studied in this experiment.

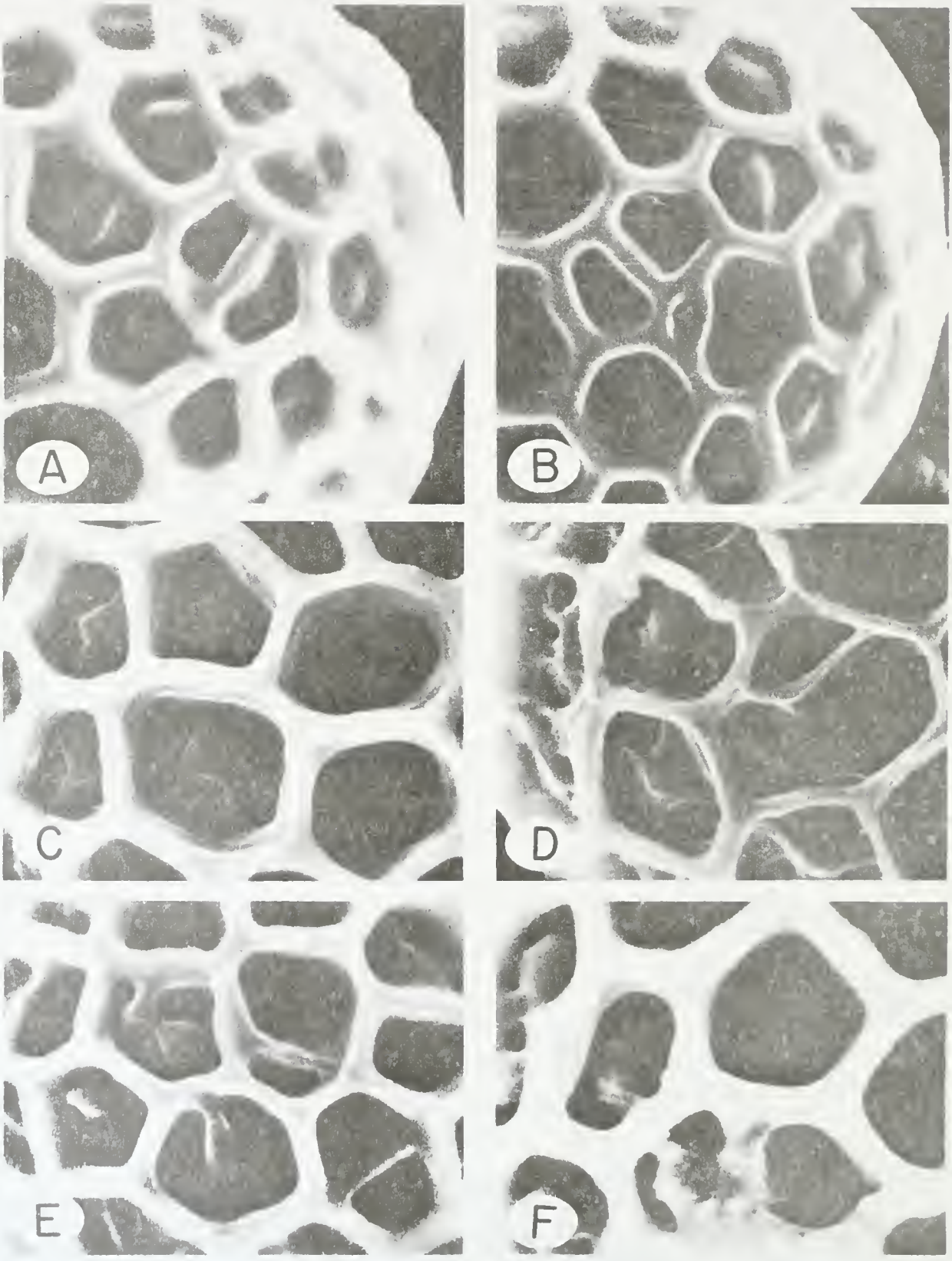


Figure 1. Scanning electron micrographs of: A. TCK spores (untreated), B. Common smut spores (untreated), C. D. E. F. TCK spores treated with laminaranase, chitinase, amyloglucosidase and cellulase plus protease, respectively.

We also tried to obtain an extracellular enzyme preparation that was capable of hydrolyzing smut spore walls by culturing Trichoderma viride on the smut spores. This omnivorous fungus grew poorly on these spores, and a suitable enzyme preparation was not obtained.

AGGLUTINATION REACTIONS

Specific glycoprotein compounds from plants are known which cause certain cells to agglutinate, i.e. bind together. These agglutinating proteins are also known as lectins, phytoagglutinins or phytohemagglutinins. These agglutinins have multiple binding sites on each molecule and are highly specific for binding certain carbohydrate moieties. Thus, if a spore wall has a carbohydrate moiety exposed which can attach to an agglutinin molecule then because it has several binding sites that agglutinin molecule will bind several cells together in a three-dimensional matrix and cause agglutination to occur. If a spore wall does not have the proper carbohydrate exposed for attachment to the agglutinin then no agglutination will occur. However, the spore wall may be partially hydrolyzed with enzymes, as indicated above, to expose new potential sites for agglutinin attachments. Five specific phytoagglutinins are available commercially, and each one is unique in the types of carbohydrates they can bind together. These reactions are analogous to the antigen-antibody reactions in humans. The agglutinins tested in this experiment included:

Agglutinin	Sugar Specificity
Wheat Germ Agglutinin	N-acetyl-glucosamine
Phytohemagglutinin-M	N-acetyl-galactosamine
Concanavalin-A	α-D-glucose and α-D-mannose
Anti-A-Lectin	?
Anti-H-Lectin	?

The reaction mixture contained 1 mg spores and 0.1, 1, or 10 mg agglutinin per ml. of buffer (0.15 M NaCl plus 0.2 M K_2HPO_4, pH 7.0). The agglutination reactions were rapid and were carried out on microflocculation ring slides at room temperature. The results were observed at 25 to 75x magnification with a microscope.

No agglutinations were observed when normal (untreated) smut spores of any type were reacted with any of the 5 agglutinins. However, agglutination did occur if the walls of the smut spores were first altered by treatment with hydrolytic enzymes. Of the many experimental reaction mixtures that were tested the most effective one for differentiating TCK from common smut spores is illustrated in Table 1.

Table 1. The smut spores were treated with cellulase for 2 hours followed by protease for 2 hours, washed thoroughly and then treated with phytohemagglutinin-M (PHA-M).

PHA-M Concentration	Agglutination Reactions	
	TCK Spores	Common Smut Spores
10 mg/ml	++++*	++
1.0	++	+
0.1	+	+

* ++++ = massive agglutination; + = trace of agglutination.

Phytohemagglutinin-M was the only agglutinin tested that had significant activity at the highest concentration tested (10 mg/ml). Hydrolytic enzyme treatments (prior to the agglutination reactions) were extended up to 48 and 72 hours, but no set of conditions were found which were superior to those described in the above table.

SPORE WALL REMOVAL WITH INORGANIC REAGENTS

In contrast to removing the spore wall in a gentle and selective manner with hydrolytic enzymes, the wall may be removed rapidly by the action of strong acids, bases and oxidizing agents. High concentrations of sulfuric acid must be used to achieve wall removal in a reasonable period of time, e.g. 18N H_2SO_4 at 22°C will remove about one-half of the reticulated wall of TCK spores in 2 hours, whereas 24N H_2SO_4 at 22°C will remove about three-fourths of this layer in the same time. Approximately 10 N NaOH has an effect similar to 18 N

H_2SO_4. Sodium hypochlorite (NaOCl), a strong oxidizing agent, is very effective in removing the cell wall, e.g. 5% NaOCl at 22°C for 1 hour caused great swelling of the spores and partial wall fragmentation. If this reaction were allowed to continue for three hours all wall and wall fragments were dissolved and only the spherical central portions of the spores remained.

Experiments were also performed in which the spores, after treatment with these strong reagents, were reacted with the agglutinating proteins, as described previously. Again, the only agglutinin that had significant activity was phytohemagglutinin-M (PHA-M) and it was active at 1 or 10 mg/ml. After 2 hours at 37°C in either 18N or 24N H_2SO_4 the spores were washed thoroughly and reacted with PHA-M to give a +++ agglutination reaction. After 2 hours at 25°C in 10N NaOH the spores gave a ++ agglutination reaction. However, the agglutination reactions of TCK and common smut spores were too similar to use this as a definitive test. No agglutination occurred if the spores were initially treated with any concentration of NaOCl.

PERIODATE REMOVAL OF SPORE WALL

Although the experiments with the strong oxidizing agent NaOCl, described above, did not lead to any useful tests, they suggested that more refined experiments with selective oxidizing agents might be fruitful. Since most of the spore wall is composed of polysaccharide

substances we needed oxidizing reagents that would selectively attack these compounds. At concentrations less than 0.2 M, sodium periodate is known to oxidize simple sugars and polysaccharides fairly specifically. At high concentrations of periodate nonspecific oxidations may occur.

Several factors are important in the periodate oxidation of polysaccharides, including: concentration of periodate, temperature, pH, time and light. Under our reaction conditions light was not an important variable, but the other four parameters were significant. Our experimental goal was to vary these four parameters until we found a set of reaction conditions that would make the TCK spores look quite different from common or grass smut spores when viewed with the light microscope or the scanning electron microscope. The initial results indicated that the periodate oxidation reaction was a very sensitive and selective way of slowly removing the cell wall of the smut spores. Furthermore it indicated that the spore walls of the common and grass smuts were being removed at a faster rate than the TCK spore walls, or that the TCK walls were thicker.

After many trials it was determined that the following conditions are near optimum for distinguishing between TCK and the other smut spores by the periodate reaction: 0.16 M Periodic Acid, 0.1 M Acetate buffer at pH 4.5, and 3 to $3\frac{1}{4}$ hours at 57^{o}C. The spore concentration in this reaction mixture should not exceed 2 mg/ml. After 3 to $3\frac{1}{4}$ hours under those reaction conditions about 95% of the grass smut spores and 75 to 90% of the common smut spores have lost their surface net-like

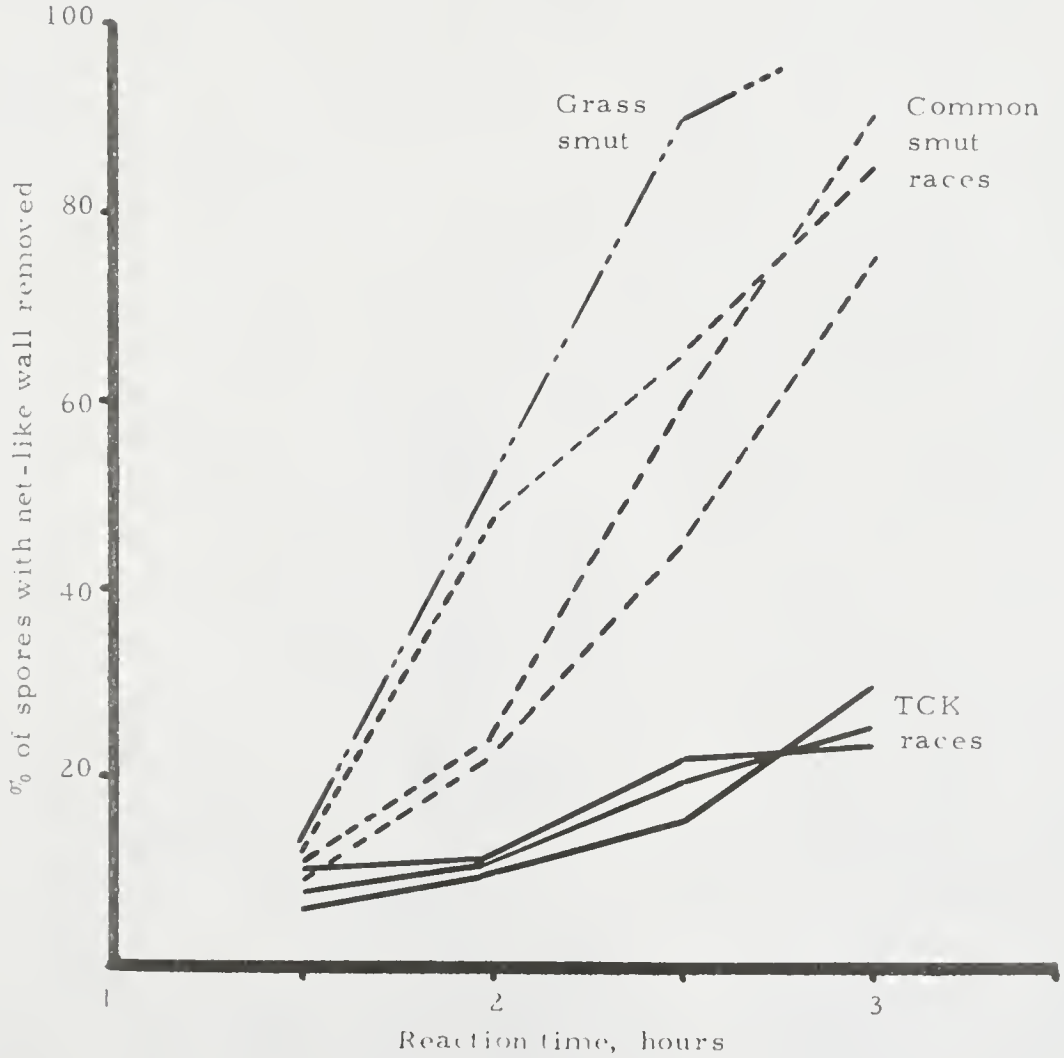
ridges and look smooth-walled whereas, only 20-25% of the TCK spores look smooth-walled, Figures 2 and 3. In addition to this large numerical difference in cell wall removal, there is also a clear microscopic difference between the net-like structures that remain on the common smut and TCK spores after this oxidation treatment. The ridges of the net-like wall that remain on the common smut spores are low and rounded, whereas those on TCK spores are long, spike-like ridges. This variation in the net-like wall that remains after the oxidation treatment can be further accentuated by the use of any of the following stains: methylene blue, rose bengal, malachite green, safranine 0, or nile blue A.

THE EFFECT OF DMSO

Following the initial success of the periodate oxidation we also conducted some preliminary experiments with another oxidizing agent that is also specific for polysaccharides. The new oxidizing agent, lead tetraacetate, was said to be just as specific but more rapid than the periodate oxidation. Our goal was to use the best of these two oxidation reactions or to use them in series and thus, shorten the time required for this test. However, we were never successful in achieving cell wall removal with the lead tetraacetate reaction.

Although cell wall oxidations were not apparent in the lead tetraacetate reaction mixtures other significant changes did occur. The cell walls of common and grass smut spores apparently became very soft and

Figure 2. The relative rates of removal of the net-like wall from TCK, common smut, and grass smut spores.



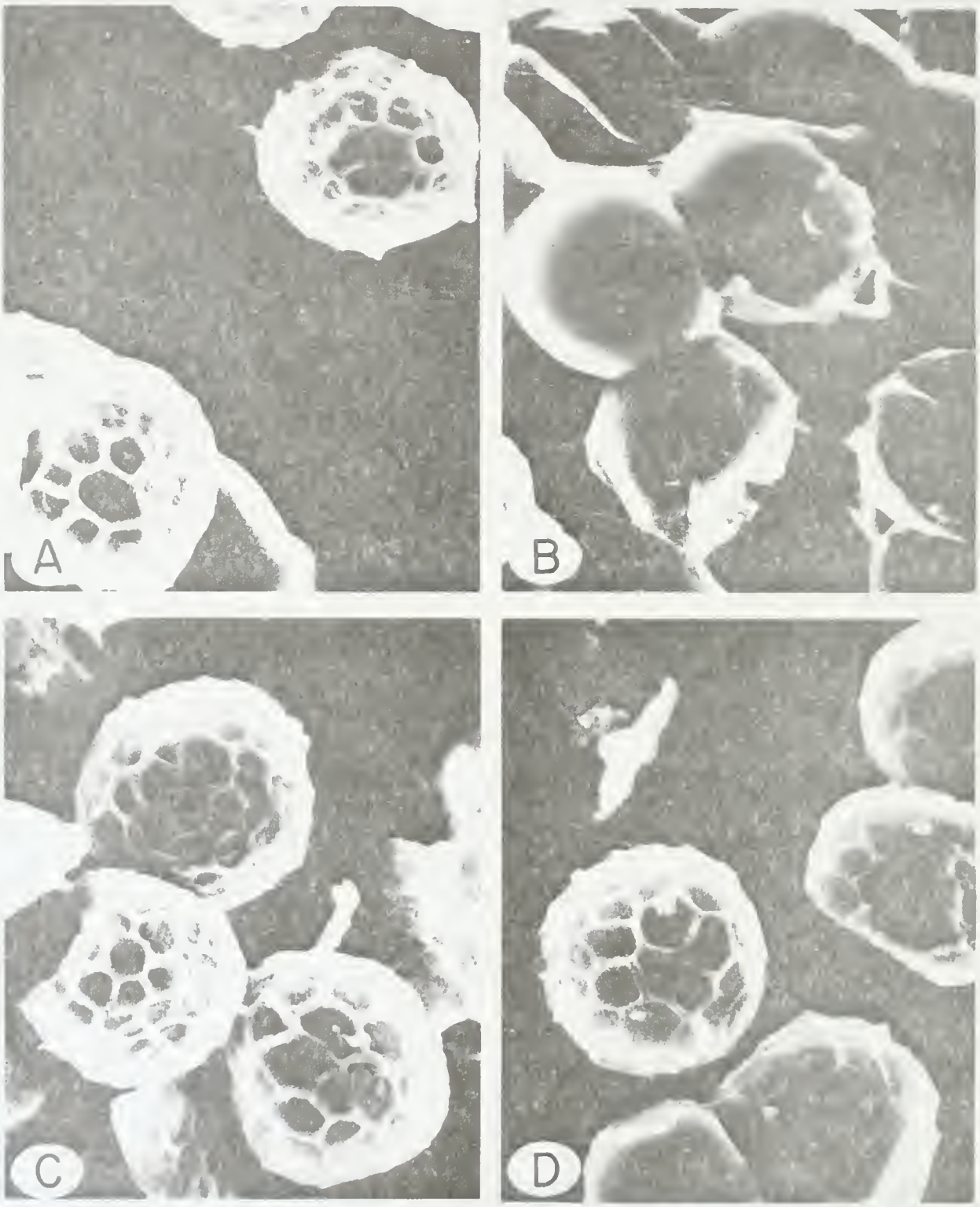


Figure 3. Scanning electron micrographs illustrating the effects of the periodate reaction. A and B. Common smut spores after 1.5 and 3 hours, respectively. C and D. TCK spores after 1 5 3 hours, respectively, in the periodate reaction mixture.

permeable. The effect of these changes was to cause the common and grass smut spores to loose their spherical form and assume a deformed, distorted or deflated appearance, Figure 4. In contrast, the TCK spores appeared unaffected by this reaction mixture. We soon learned that the chemical responsible for this deformation was not the oxidizing agent lead tetraacetate but one of the solvents in the reaction mixture, dimethylsulfoxide, commonly known as DMSO. Dimethylsulfoxide is a very effective solvent for many organic compounds and has the capacity to penetrate cell walls very quickly. After studying the effects of concentration, temperature and time, on this deformation reaction, it became evident that pure DMSO at room temperature for 10 minutes gave the maximum effect. It is interesting to note that another very potent organic solvent, N, N-dimethylformamide, has an effect similar to DMSO on smut spores.

NEGATIVE STAINING OF SPORE CAPSULE

There is some variation in the existence of a capsule and the thickness of the capsule on the outside of the smut spores, Figure 5. Although we have not yet examined all collections of these smut spores, our observations presently indicate that the capsule is more likely to be present and to be much thicker on TCK spores than on common or grass smut spores. This capsule, however, is usually difficult to

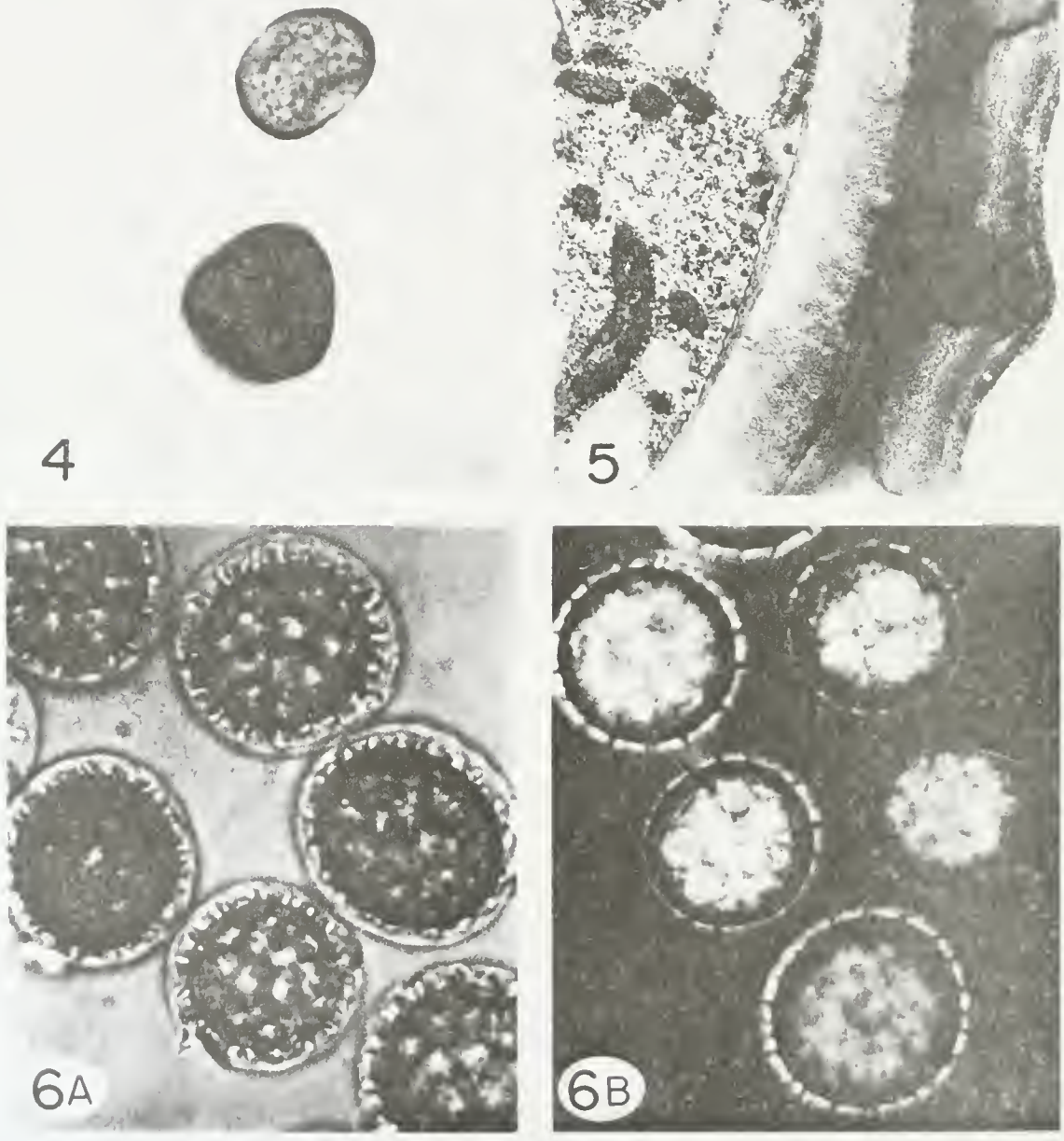


Figure 4. Effect of DMSO on common smut spores.

Figure 5. Transmission electron micrograph of common smut spore showing outer capsular material between the net-like ridges of the cell wall. This figure is by courtesy of Prof. W. M. Hess, Brigham Young University.

Figure 6. TCK spores showing the capsule, A. untreated, B. negative stain technique.

observe. The capsular material apparently is so fine that it is transparent to the electron beam of the scanning electron microscope and it is also transparent to the light beam of the light microscope. Therefore, to utilize this aspect of the spore morphology as a distinguishing characteristic a method had to be developed to make it easier to observe the capsule.

Smut spores in a drop of water can be mixed with a drop of India Ink (colloidal suspension of carbon particles) on a glass slide and observed under the microscope at 400 to 1000X magnification. The carbon particles will block the light path, however, since the carbon particles cannot penetrate the capsule and the capsule is transparent to light, the capsules appear as halos around the spores. This is not only an easy and efficient method of detecting the presence and thickness of the capsule but also permits observation of the characteristic net-like surface of the spores, Figure 6. The raised net-like surface of TCK spores appear as long, sharp, spines, in a median view, in contrast to the shorter and blunter ridges of the common smut spores.

Precise determinations on the thickness of the capsule on all TCK and common smut races and grass smut species is a project that should be carried out, for it appears that this technique can be developed into a suitable test to use in characterizing these smut spores.

FLUORESCENCE AND ULTRAVIOLET SPECTROPHOTOMETRY

Methanol extracts were prepared from TCK and common smut spores and studied with the fluorescence and ultraviolet spectrophotometers. The results of these studies indicated that these methods are not sensitive or specific enough to be useful for differentiating the smut species.

MASS SPECTROSCOPY

The mass spectrometer is commonly used as an analytical tool in organic chemistry for identifying unknown compounds. By using a direct inlet probe, a small number of smut spores can be placed directly into the ion beam of the mass spectrometer and the compounds present in the spores analyzed in a relative manner. That is, the recorded printout of the analysis will not permit the chemical identification of the compounds present, for the mixture present in the spores will be too complex. It was hypothesized, however, that the printout for TCK spores would be sufficiently different from the other smut spores to permit identification of smut species.

Up to the present time we have only been able to make 5 analyses using this technique: three analyses on TCK spores and two analyses on common smut spores. The results are encouraging and the printouts have been sharp and clear. With this small number of samples, however,

we have not yet determined the optimum operating conditions for the instrument. We believe that this is a very sensitive and specific method that can be developed with further effort.

GERMINATION CHARACTERISTICS

If several hundred or more smut spores are available for testing and if the results are not needed immediately it is often advisable to check the germination characteristics of the spores. If the spores will germinate in one week on water agar at 17°C they are definitely not TCK spores. A few common smut races and grass smut species require a colder temperature and longer than one week to germinate, but the crucial point is that no TCK spores will germinate at 17°C. Therefore, if no germination occurs in one week we cannot be sure that the spores being tested are TCK, but if the spores do germinate at 17°C they are definitely not TCK spores.

SUMMARY AND DISCUSSION

1. As a result of the experience we have had in the past ten months in developing methods for the identification of TCK spores, five tests can now be described to aid in the characterization of TCK spores. These recommended methods are described in the appendix of this report.

It is possible to gather information from all five recommended methods if 500 or more spores are available for study. At the other extreme, if only 5 to 10 spores are available for study the analyst is limited to two tests, the negative staining method and the DMSO method. When a sufficient number of spores is available we advocate that these tests be used to complement each other for confirmation of spore identification, instead of applying only one or two of the easiest methods.

2. Most of the tests described herein were developed using three races of TCK spores, 3 races of common smut spores and one species of grass smut. Since there are about 17 races of TCK, 30 races of common smut and 5 to 10 species of grass smuts known in the Pacific Northwest it is imperative that all of these races be gathered together and tested by these recommended methods. Until that is done, the variation within each smut species will remain unknown and thus cast a doubt on the validity of any test or group of tests.

3. The typical procedure used in monitoring wheat shipments for smut spores is to take a 50 gram sample of seed, add water, shake, and collect the fine particulate matter either by centrifugation or filtration. In a washed preparation of this type, smut spores often represent only a small fraction of the particulate matter found in the concentrated sample. Since some of the recommended tests (e.g. agglutination and periodate oxidation) require a farily clean spore suspension, the contaminating debris must be removed. We haven't yet developed a precise

procedure for cleaning-up these spore preparations but suggest that filtrations through several fine nylon filters (e.g. 200, 100, 50 micron mesh) will remove most of the debris quickly. If all of the debris must be removed, e.g. in the mass spectroscopy method, the filtered spore preparation should be purified by a technique such as density-gradient centrifugation.

4. The methods that have been described in this report may be of value not only to seed inspectors and scientists studying smut spores but also to others studying similar thick-walled fungal spores. Therefore, the data we have collected will be written up in more detail and submitted for publication in Phytopathology, an international journal of plant diseases. We may also condense it and have it published in an applied seed-testing journal.

5. Although the tests described in this report will certainly aid in the identification of TCK spores, additional research on improving these tests and developing even more specific and more sensitive tests are recommended. Our suggestions for future research are as follows:

a) All races and species of smut fungi that can be confused with TCK spores should be gathered together and tested by the methods recommended in this report.

b) A specific clean-up procedure should be developed that will remove all contaminating debris from the smut spores in a water-wash preparation from wheat seeds.

c) Two new highly specific and sensitive methods should be studied in detail. The first is the mass spectroscopy method which is described briefly in the main section of this report. The second is an immunological method in which antibodies are prepared that are highly specific for each spore type. These specific antibodies are then tagged, either with a radioactive tracer or with a fluorescent tag to aid in the positive identification of smut spores when only 5 to 10 spores are available. Both of these methods would probably be much more specific and sensitive than any of the methods yet developed.

APPENDIX

RECOMMENDED TESTS

Periodate Removal of Spore Wall

Place a sample of the smut spores (less than 2 mg/ml) into a solution containing 0.16 M Periodic Acid, 0.1 M Acetate Buffer at pH 4.5. Incubate with occasional stirring at 57°C for 3.25 hours. Collect spores by filtration or centrifugation, stain lightly with methylene blue (10 mg/100 ml water, immediate reaction), and view at about 1000X magnification. The great majority of the common and grass smut spores will have smooth walls, whereas most of the TCK spores will still show prominent spine-like ridges on the outer spore surface when seen in a median view.

Negative Staining of Spore Capsule

A few smut spores (previously stained with methylene blue) in a drop of water are mixed with a drop of India Ink on a glass slide. Apply a cover slip and view at 400X to 1000X magnification. Observe presence of capsule, thickness of capsule, the length and shape of the spine-like ridges on the outer surface of the spores, and the overall diameter of the spore.

Spore Deformation Caused by DMSO

Place smut spores into a small volume of pure dimethylsulfoxide (DMSO). Stir gently several times. Place mixture on a glass slide, apply a cover slip and view at 400 to 1000X magnification. TCK spores remain spherical and normal in appearance, whereas common and grass smut spores appear collapsed and deformed.

Germination Characteristics

Seed the spores to be tested on water agar (3 gm agar/100 ml) in Petri dishes. Incubate at 17°C for one week and then observe at 25 to 75X magnification. If any of the spores have germinated they are not TCK spores.

Agglutination Reactions

In all of these reaction mixtures the spore concentration should not exceed 1 mg/ml buffer. Place smut spores in the following enzyme solution and then incubate, with gentle agitation, at 37°C for 2 hours: #C-7377 Cellulase 1 mg/2 ml buffer (0.1 M sodium acetate, pH 5.0). Carefully wash spores with buffer and recover spores with the aid of Millipore Filters, 10μ pores. Place spores in the following enzyme solution and then incubate, with gentle agitation, at 37°C for 2 hours: #P-5130 Protease 1 mg/2 ml buffer (0.1 M phosphate, pH 7.0). Cellulase and protease were purchased from Sigma Chemical Company. Wash

with phosphate buffer and recover spores as before. Place spores in a reaction mixture containing 10 mg phytohemagglutinin-M (Calbiochem. Corp. #526511) per ml of buffer (0.15 M NaCl plus 0.2 M K_2HPO_4, pH 7.0). The agglutination reaction is rapid and may be carried out and viewed on microflucculation ring slides at 50X magnification. TCK spores give a very strong agglutination reaction, whereas common smut spores exhibit a weak reaction.

TASK 4

A STUDY TO DISCOVER AND IDENTIFY A LOW COST CHEMICAL TREATMENT WHICH WILL ERADICATE OR ARREST THE DEVELOPMENT OF DWARF BUNT

Supported by

The Old West Regional Commission
and
The Montana Wheat Research and Marketing Committee

Submitted by

W. G. Dewey, Professor of Plant Breeding

J. O. Evans, Research Assistant

Plant Science Department

J. A. Hoffmann, Research Plant Pathologist

ARS-USDA, Crops Research Laboratory

Utah State University, Logan, Utah

FINAL REPORT

I. Project Title.

A Study to Discover and Identify a Low Cost Chemical Treatment which will Eradicate or Arrest the Development of Dwarf Bunt.

II. Project Number.

7504

III. Beginning and Ending Dates for the Contract Period.

January 1, 1975 to June 30, 1976

IV. Activities, Results, and Conclusions.

Three approaches to the chemical control of dwarf bunt of winter wheat were investigated:

(1) The use of spring foliar sprays to eradicate the dwarf bunt fungus once it has become established in the wheat plant.

(2) The use of seed treatment fungicides, particularly those with systematic action, to prevent the initial infection and/or establishment of the fungus in the wheat seedlings.

(3) The use of fall soil sprays to eradicate the fungus spores at or near the soil surface, where most infection takes place.

Foliar Spray Studies.

Foliar applications of a number of known or suspected systemic fungicides were made to dwarf bunt infected wheat plants in the spring of 1975 and again in 1976. In 1975 seven fungicides were applied at two rates (the manufacturer's recommended rate and double this rate) and at three dates. The chemicals, rates, dates and results are summarized in Table 1.

In 1976, 11 fungicides were applied at three rates (the manufacturer's recommended rate and two and four times this rate) and at two dates. The chemicals, rates, dates, and results for this study are shown in Table 2.

The artificial inoculation procedures were very successful in both years, with infection levels in the check plots generally in the 75-100% range. None of the chemicals significantly reduced infection at any of the rates or dates of application in either year, in spite of the fact that rates were sufficiently high as to result in phytotoxicity in several instances.

It would appear that until and unless new systemic fungicides become available with a greater capability of differentially affecting the fungus and the wheat host, that this approach to dwarf bunt control is not particularly promising.

Seed-Treatment Studies.

Two types of seed treatment tests were conducted during the winter wheat crop year 1975-76. One involved three varieties, representing low, intermediate, and high levels of genetic resistance. Three of the most promising seed-treatment fungicides were applied, each at three rates. Three dates of seeding were used and the test was replicated at several locations in Utah, Idaho, Montana, Washington, and Colorado. The purpose of this study was to determine the most effective and economical combination of fungicide, varietal resistance, and planting date. The findings are summarized in Table 3.

The second seed-treatment study involved the screening of a relatively large number of chemicals for their possible effectiveness against dwarf bunt. A single susceptible wheat variety was used in this test. Sixteen chemicals, each at three rates, were used. Plantings were made at six locations; however, the plots at Colville, Washington and Craig, Colorado were abandoned because of winter injury and low levels of infection. The results of this study are shown in Table 4.

Since we have only one year's data on this seed-treatment work, the conclusions are somewhat tentative. However, it appears that TBZ

(thiabendazole), at the 2 and 4 oz. per bushel rates provides fair-to-good dwarf bunt control at most locations. Some location-to-location variation in effectiveness was noted, however. Uniroyal's H-719 showed some promise at the 4 oz. per bushel rate. Mid-September or mid-October seeding seemed to result in less dwarf bunt than did planting around the first of October. The combination of at least a fair level of varietal resistance, coupled with TBZ seed treatment appears to constitute an effective deterrent to dwarf bunt.

Soil Spray Studies.

Fifteen compounds were sprayed onto the soil surface of artificially-inoculated plots of a susceptible winter wheat variety at Logan, Utah in the fall of 1975. The chemicals, rates, and the resulting infection percentages are listed in Table 5. H-719 and CGA 39896 were especially effective at the higher rates of application. Since no effort was made to protect the plants from the soil spray, there is a question as to whether the fungicide was acting against the spores in the soil or as a systemic protectant within the plant.

Most of the compounds which showed evidence of significant dwarf bunt control in the above studies are still in the development and testing stages; consequently, meaningful cost figures on a commercial scale are not available.* Judging from the relative amounts of material involved

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*Merck & Co., the manufacturer of TBZ, has recently announced that its registration for wheat has been approved. Cost figures are still not definite, however.

to achieve adequate control, however, seed treatment will likely prove more economically feasible than will the soil sprays.

V. Future Directions.

The first year-and-a-half of investigations covered by this contract have resulted in some interesting and beneficial leads which should ultimately aid in bringing the dwarf bunt problem under control. The findings of these studies will be continued and expanded under a new contract administered by the Pacific Northwest Regional Commission and the Oregon Department of Agriculture.

VI. Financial Information.

An expenditure summary is appended.

Principal Investigator: Wade G. Dewey

Wade G. Dewey

TABLE 1. Percent Dwarf Bunt in Cheyenne Wheat at Logan Receiving 1975 Spring Foliar Spray Treatments of Systemic Fungicides + Surfactants.

			% infection* when treated on given date		
% Surfactant	Fungicide	Rate (ai/acre)	April 2	April 21	May 15
None	Untreated Check	- - -	78	90	83
"	carboxin	32 oz	75	90	83
"	"	64 oz	74	84	80
"	benomyl	32 oz	83	93	74
"	"	64 oz	79	90	82
"	thiabendazole (a)	32 oz	81	94	85
"	"	64 oz	91	92	83
"	Bay Meb 6447	4 oz	85	94	83
"	"	8 oz	78	86	85
"	CGA 39896	8 oz	83	83	88
"	"	16 oz	86	91	87
"	RH 124	4 oz	84	92	84
"	"	8 oz	78	83	80
"	EL 222	15 gm	82	93	84
"	"	30 gm	74	90	90
5.0% DMSO	Untreated check	- - -	84	92	87
"	carboxin	32 oz	84	90	84
"	"	64 oz	69	90	89
"	benomyl	32 oz	74	91	88
"	"	64 oz	79	92	75

continued

TABLE 1. continued.

% Surfactant	Fungicide	Rate (ai/acre)	% infection* when treated on given date		
			April 2	April 21	May 15
5.0% DMSO	thiabendazole (a)	32 oz	90	90	88
"	"	64 oz	90	89	79
"	Bay Meb 6447	4 oz	93	89	87
"	"	8 oz	79	90	84
"	CGA 39896	8 oz	75	79	83
"	"	16 oz	89	83	89
"	RH 124	4 oz	86	85	76
"	"	8 oz	85	89	89
"	EL 222	15 gm	80	83	77
"	"	30 gm	92	86	82
0.5% X-77	Untreated check	- - -	92	79	85
"	carboxin	32 oz	84	93	77
"	"	64 oz	89	91	76
"	benomyl	32 oz	72	92	90
"	"	64 oz	89	90	78
"	thiabendazole (a)	32 oz	82	90	79
"	"	64 oz	79	82	90
"	Bay Meb 6447	4 oz	86	89	80
"	"	8 oz	76	81	84
"	CGA 39896	8 oz	83	89	77
"	"	16 oz	56	89	84
"	RH 124	4 oz	86	95	80
"	"	8 oz	67	84	84
"	EL 222	15 gm	71	89	82
"	"	30 gm	81	84	72

continued

TABLE 1. continued.

% Surfactant	Fungicide	Rate (ai/acre)	% infection* when treated on given date		
			April 2	April 21	May 15
0.5% Tronic	Untreated check	- - -	80	90	77
"	carboxin	32 oz	89	90	91
"	"	64 oz	82	92	80
"	benomyl	32 oz	85	90	81
"	"	64 oz	73	87	82
"	thiabendazole (a)	32 oz	86	90	83
"	"	64 oz	73	86	80
"	Bay Meb 6447	4 oz	88	90	85
"	"	8 oz	91	91	87
"	CGA 39896	8 oz	82	93	86
"	"	16 oz	85	93	87
"	RH 124	4 oz	81	84	80
"	"	8 oz	80	87	87
"	EL 222	15 gm	82	90	87
"	"	30 gm	83	92	77

* averaged over three replications

TABLE 2. Percent Dwarf Bunt in Cheyenne Wheat at Logan Receiving 1976 Spring Foliar Spray Treatments of Systemic Fungicides + Surfactant.

% Surfactant	Fungicide	Rate (ai/acre)	% infection* when treated on given date	
			April 19	May 10
None	Untreated check	- - -	99	99
"	carboxin	32 oz	99	99
"	"	64 oz	98	98
"	"	128 oz	100	99
"	benomyl	32 oz	98	100
"	"	64 oz	99	99
"	"	128 oz	100	99
"	thiabendazole (a)	32 Oz	99	98
"	"	64 oz	99	99
"	"	128 oz	99	100
"	H 719b	4 oz	99	98
"	"	8 oz	99	97
"	"	16 oz	98	99
"	Bay Meb 6447	4 oz	99	99
"	"	8 oz	98	98
"	"	16 oz	100	99
"	Bay Meb 6449	4 oz	99	99
"	"	8 oz	99	98
"	"	16 oz	99	99
"	RH 124	4 oz	99	99
"	"	8 oz	99	99
"	"	16 oz	99	99
"	RH 2161	4 oz	99	99
"	"	8 oz	98	99
"	"	16 oz	99	98

continued

TABLE 2. continued.

% Surfactant	Fungicide	Rate (ai/acre)	% infection* when treated on given date	
			April 19	May 15
None	EL 222	15 gm	100	99
"	"	30 gm	100	98
"	"	60 gm	99	97
"	EL 228	60 gm	98	98
"	"	120 gm	99	99
"	"	240 gm	100	99
"	BAS 317 10 F	200 gm	99	100
"	"	400 gm	99	99
"	"	800 gm	100	99
1.0% Tronic	Untreated check	- - -	100	99
"	carboxin	32 oz	99	99
"	"	64 oz	98	99
"	"	128 oz	99	99
"	benomyl	32 oz	99	98
"	"	64 oz	98	100
"	"	128 oz	99	99
"	thiabendazole (a)	32 oz	99	97
"	"	64 oz	98	99
"	"	128 oz	99	99
"	H 719b	4 oz	99	100
"	"	8 oz	99	99
"	"	16 oz	99	98
"	Bay Meb 6447	4 oz	99	99
"	"	8 oz	100	99
"	"	16 oz	98	99

continued

TABLE 2. continued.

% Surfactant	Fungicide	Rate (ai/acre)	% infection* when treated on given date	
			April 19	May 10
1.0% Tronic	Bay Meb 6449	4 oz	99	99
"	"	8 oz	98	98
"	"	16 oz	99	99
"	RH 124	4 oz	99	99
"	"	8 oz	99	99
"	"	16 oz	99	98
"	RH 2161	4 oz	100	99
"	"	8 oz	99	98
"	"	16 oz	99	98
"	EL 222	15 gm	99	98
"	"	30 gm	99	99
"	"	60 gm	99	99
"	EL 228	60 gm	99	99
"	"	120 gm	98	99
"	"	240 gm	98	99
"	BAS 317 10 F	200 gm	99	98
"	"	400 gm	99	99
"	"	800 gm	99	99

* averaged over 3 replications

TABLE 3. Percent Dwarf Bunt in 3 Wheat Varieties Treated with Systemic Fungicides in 1975-76.

			% infection at given locations and seeding dates*								
			Kalispell	Logan		Blue Creek			Preston		
Variety	Fungicide	Rate (oz/bu)	Oct. 1	Oct. 1	Oct. 15	Sept. 16	Oct. 2	Oct. 16	Sept. 17	Oct. 3	Oct. 17
Hansel	benomyl	0	1.375	0.125	0.875	0.375	0.25	0.50	1.25	0.125	0.375
"	"	2	0.50	0.0	0.0	0.0	0.125	0.0	0.0	0.125	0.0
"	"	4	0.375	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	"	8	0.625	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	thiabendazole (a)	0	0.875	0.125	0.75	0.875	0.25	0.0	0.50	0.250	0.875
"	"	2	0.125	0.0	0.0	0.0	0.0	0.0	0.0	0.125	0.0
"	"	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	"	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	H 719a	0	1.125	0.125	0.625	1.125	0.125	0.875	1.25	0.125	1.125
"	"	2	0.875	0.0	0.0	0.0	0.0	0.0	0.0	0.125	0.0
"	"	4	0.125	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
"	"	8	0.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

continued

TABLE 3. continued.

Variety	Fungicide	Rate (oz/bu)	% infection at given locations and seeding dates*								
			Kalispell	Logan		Blue Creek			Preston		
			Oct. 1	Oct. 1	Oct. 15	Sept. 16	Oct. 2	Oct. 16	Sept. 17	Oct. 3	Oct. 17
Ranger	benomyl	0	6.00	8.00	13.50	1.875	2.75	1.75	11.25	6.25	8.75
"	"	2	6.50	3.75	6.00	0.375	1.25	1.00	5.25	4.00	3.75
"	"	4	3.875	0.75	2.75	0.250	0.375	0.625	4.75	3.25	3.00
"	"	8	1.50	0.75	1.00	0.00	0.375	0.625	3.50	2.75	0.75
"	thiabendazole (a)	0	6.75	16.25	15.25	3.25	2.00	3.00	5.50	9.50	7.50
"	"	2	0.125	1.75	0.25	0.25	0.75	0.50	2.00	4.25	1.50
"	"	4	0.125	0.75	0.25	0.375	0.125	0.625	1.00	0.25	0.50
"	"	8	0.00	0.00	0.25	0.125	0.00	0.25	0.625	0.75	0.125
"	H 719a	0	2.875	12.75	22.75	2.50	3.25	4.75	10.75	9.75	5.25
"	"	2	2.50	6.50	11.50	0.50	0.625	1.00	3.50	8.75	2.375
"	"	4	2.25	1.25	4.50	0.00	0.375	0.25	2.25	7.00	0.875
"	"	8	0.125	0.75	1.25	0.00	0.125	0.00	0.75	3.25	0.00

continued

TABLE 3. continued.

Variety	Fungicide	Rate (oz/bu)	% infection at given locations and seeding dates*								
			Kalispell	Logan		Blue Creek			Preston		
			Oct. 1	Oct. 1	Oct. 15	Sept. 16	Oct. 2	Oct. 16	Sept. 17	Oct. 3	Oct. 17
Wanser	benomyl	0	65.75	95.00	94.00	88.25	94.50	84.25	68.75	70.00	53.75
"	"	2	52.50	70.75	41.25	70.00	82.00	47.25	55.00	38.75	20.00
"	"	4	35.00	47.25	31.25	60.00	61.25	31.50	35.00	25.00	9.00
"	"	8	38.00	30.25	9.75	42.50	37.25	14.00	25.00	31.25	9.00
"	thiabendazole (a)	0	60.50	97.50	95.50	85.75	92.75	86.00	76.25	78.75	62.50
"	"	2	6.00	37.75	8.75	20.50	40.00	12.25	47.50	48.75	33.75
"	"	4	9.25	28.75	9.00	16.25	16.25	7.00	8.75	11.25	7.00
"	"	8	0.50	2.75	1.25	8.25	6.75	1.00	1.125	3.25	2.75
"	H 719a	0	56.25	94.875	95.75	86.50	93.25	85.75	70.00	71.25	55.00
"	"	2	58.75	86.00	35.25	66.50	80.00	23.75	56.25	55.00	25.00
"	"	4	38.75	76.75	27.75	51.25	64.00	15.50	38.75	45.00	8.75
"	"	8	32.25	62.75	10.00	31.25	38.50	7.00	19.25	25.00	1.25

continued

TABLE 3. continued.

* averaged over the 4 replications

LSD (.05) = 0.49 for Hansel on all 3 dates of seeding at Blue Creek and Preston.

LSD (.05) = 3.19 for Ranger on all 3 dates of seeding at Blue Creek and Preston.

LSD (.05) = 16.21 for Wanser on all 3 dates of seeding at Blue Creek and Preston.

LSD (.05) = 0.49 for Hansel on early October date of seeding at all 4 locations.

LSD (.05) = 6.93 for Ranger on early October date of seeding at all 4 locations.

LSD (.05) = 21.16 for Wanser on early October date of seeding at all 4 locations.

LSD (.05) = 13.05 for all 3 varieties on early October date of seeding at all 4 locations.

LSD (.05) = 21.33 for all 3 varieties on later 2 dates of seeding at all 4 locations.

LSD (.05) = 19.46 for all 3 varieties on all 3 dates of seeding at Blue Creek and Preston.

TABLE 4. Percent Dwarf Bunt in Wanser Wheat Receiving Systemic Fungicide Seed Treatments.

Treatment	Rate (oz ai/bu)	% infection							
		Logan		Blue Creek		Preston		Kalispell	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Untreated check	- - -	80	85	80	80	75	79	70	60
Bay Meb 6447	1	70	70	75	68	85	90	70	75
"	2	4	9	60	70	70	60	55	29
"	4	0	9	55	65	40	6	1(PS)	30
Bay Meb 6449	1	45	40	55	75	78	75	75	60
"	2	37	28	65	70	75	70	60	65
"	4	0.5	3	65	65	70	65	55	30
benomyl	1	17	11	55	60	68	80	75	65
"	2	2	2	45	35	70	80	17	60
"	4	blank(4)	4	13	30	65	55	27	16
thiabendazole (a)	1	blank(3)	0	45	50	55	45	10	2
"	2	0	0.5	25	2	50	30(PS)	0	3
"	4	0	0	0	0	10	7(PS)	4	2

continued

TABLE 4. continued.

Treatment	Rate (oz ai/bu)	% infection							
		Logan		Blue Creek		Preston		Kalispell	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
thiabendazole (b)	1	0.5	0.5	60	50	60	40	16	17
"	2	0	0	13	21	35	6(PS)	5	6
"	4	0	0	0	0	20	12(PS)	0.5	0
ME 126	1	90	70	35	55	80	38	60	60
"	2	85	75	67	65	70	65	70	60
"	4	45	70	60	45	55	35	65	75
RH 2161	1	75	55	75	60	65	55	60	60
"	2	35	2	68	60	50	75	40	55
"	4	0	0(PS)	55	50	68	65	0(PS)	28(PS)
RH 124	1	87	85	65	55	67	65	25	50
"	2	95	80	75	65	70	50	65	65
"	4	65	80	50	60	60	60(PS)	65	65

continued

TABLE 4. continued.

Treatment	Rate (oz ai/bu)	% infection							
		Logan		Blue Creek		Preston		Kalispell	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Untreated check	- - -	75	75	65	45	70	70	67	70
carboxin	1	80	80	65	50	55	70	75	75
"	2	90	87	70	65	75	80	68	35
"	4	75	70	60	40	75	80	90	65
carboxin + PPG 124	1	blank(66)	65	48	39	70	75	85	65
"	2	blank(52)	50	45	60	60	65	50	50
"	4	20	9	45	19	30	60	55	20(PS)
H 719a	1	22	20	45	33	70	68	90	75
"	2	9	7	37	9	65	60	50	58
"	4	1	blank(2)	14	3	28	40	16	45
UBI 1159	1	90	blank(74)	60	65	60	65	67	70
"	2	80	55	60	50	65	75	70	68
"	4	55	65	47	35	65	65	75	75

continued

TABLE 4. continued.

Treatment	Rate (oz ai/bu)	% infection							
		Logan		Blue Creek		Preston		Kalispell	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
UBI 1160	1	80	70	60	65	55	68	85	70
"	2	95	80	65	65	68	65	75	38
"	4	80	75	65	60	65	68	75	68
UBI 1161	1	70	95	70	60	70	70	70	68
"	2	60	40	75	55	75	80	75	65
"	4	50	22	70	50	75	65	60	70
CGA 39896	1	75	87	60	40	55	70	90	60
"	2	70	80	78	40	60	60	70(PS)	18(PS)
"	4	75(PS)	85(PS)	70	55	65(PS)	75	86(PS)	12(NS)

PS = partial stand due to phytotoxicity

NS = no stand due to phytotoxicity - data point generated using STATPAC/GLSC

blank = this row not planted - data point generated using STATPAC/GLSC

LSD (.05) = 28.88

TABLE 5. Percent Dwarf Bunt in Wanser Wheat at Logan Receiving 1975-76 Fungicide Soil Sprays.

Fungicide	% infection* at given rate (lbs ai/acre)			
	0	1	2	4
PCNB	88	82	40	29
HCB	86	49	30	13
thiram	89	76	55	53
maneb	84	64	40	23
thiabendazole (a)	83	63	42	19
H 719	89	46	12	1
carboxin	85	84	65	56
benomyl	82	53	31	15
Bay Meb 6447	82	71	64	24
Bay Meb 6449	85	71	58	33
CGA 39896	89	38	4	0
EL 222	88	82	75	63
RH 124	81	79	83	81
RH 2161	75	62	54	49
UBI 1159**	80	58	36	8

LSD (.05) = 22.76 for rate within each fungicide.

* averaged over 3 replications

** H 719/mancozeb in 1/5 ratio

TASK 5

NATURE OF INFECTION BY TILLETIA CONTROVERSA AND GENETIC AND HISTOLOGICAL ASPECTS OF RESISTANCE

Supported by

The Old West Regional Commission
and
The Montana Wheat Research and Marketing Committee

Submitted by

R. Duran, Plant Pathologist

G. W. Bruehl, Research Assistant

J. F. Schafer, Chairman

Plant Pathology Department

R. F. Line, USDA, ARS

J. R. Waldher, USDA, ARS

ARS-USDA, Crops Research Labroatory

Washington State University, Pullman, Washington

Preliminary Studies:

Prior to the start of this research, it was necessary to select a reliable histological staining schedule which would differentially stain infecting smut hyphae from the wheat host tissue. Wheat inoculated with the common bunt fungus, Tilletia caries, was used in this study. Several well known staining schedules, and modifications thereof, were tested and all proved unreliable or inadequate for the purposes of the project. An "IKI-crystal violet" schedule was developed which proved superior to all others tested. This schedule clearly demonstrates smut hyphae in host tissue and is now used routinely.

Field Studies:

These were undertaken to determine the following under field conditions, 1) when T. controversa infects wheat, 2) the site on the wheat plant where the fungus gains entry, and 3) any histological expression of resistance.

The following wheat varieties were sown in a plot located near Colville, Washington on October 1, 1975: Nugaines, Orin, Wanser, Westmont, Requa, Jeff, Luke, and Hohenheimer. The first four varieties are susceptible to the race of T. controversa used in this experiment and the last four exhibit varying degrees of

resistance. Soil was infested with teliospores of the fungus at the rate of ca. 1g/row.

Beginning November 15, 1975 and at two-week intervals thereafter, 10 plants of each variety were sampled and examined for the presence of smut hyphae. Sampling continued until March, 1976 and up to that time no trace of infection was observed. Sampling was suspended at this time due to the poor physical condition of the plot which had developed by that time. Repeated disruption of the snow cover on the plot was unavoidable during sampling. This resulted in premature melting of the snow, subsequent freezing of the water, and the formation of an ice cover over the plot. Sampling was resumed in June, 1976. The remaining stand was limited and plants of some varieties were in insufficient amounts for further sampling. However, mycelium of T. controversa was observed in the developing heads of the variety "Wanser." It therefore appears that infection may occur as late as March or April. Insufficient data were available from this experiment to determine the site of entry or the histological expressions of resistance.

Laboratory and Greenhouse Studies:

Wheat with known bunt resistance factors (Bt genes) were inoculated with a known race of T. controversa (Race D-2) and examined at various states of development to determine any

histological expression of resistance.

Several inoculation procedures were studied to determine which best suited this investigation. 1) Wheat was grown to the "2-leaf" stage and sprayed with a suspension of germinating teliospores of T. controversa. 2) Wheat seedlings were grown through a layer of agar covered with germinating teliospores. 3) Seed was mixed with an inoculum suspension and then sown in vermiculite. 4) Germinating seeds were treated as in #3. 5) Plants were "spot-inoculated" on the coleoptile shortly after germination and then seeded in vermiculite. In all these treatments, plants were suitably incubated and sampled at various intervals for histological examination. Only the last procedure gave a high percentage of infection with adequate inoculum density to facilitate penetration studies, and did not damage the young plants. This method was therefore chosen for further studies.

The following varieties were inoculated with race D-2 of T. controversa: Red Bobs, Requa, Nugaines, and PI 178383. They vary from highly susceptible to highly resistant in the order they are given. The results recorded are as follows:

Red Bobs (highly susceptible)

Penetration of the coleoptile occurs in about 10 days following inoculation and the fungus then grows rapidly toward the growing point via the space between the coleoptile and the first leaf. Within 30 days the fungus has advanced downward and laterally and

and is found in the 1st and 2nd leaves as well as the leaf bases and tissues just below the growing point. It is occasionally found in the tillar initials. In all cases the mycelium is intercellular, never forming hausotria or absorbtive structures of any kind. After 50 days, the fungus has further spread through leaf tissues and in the tissues just below the growing point. In a few plants, the head primordia of both the main culm and tillers have been invaded. After 90 days most head primordia are infected and mycelium is evident in nearly all leaf tissues.

Requa (mildly resistant)

Mycelial progress in this variety is almost identical to that in Red Bobs. Its resistance mechanism probably functions at a later stage in development, i.e., in the nearly mature and infected heads.

Nugaines (resistant)

Progress in this variety is similar to that of Red Bobs, although fewer plants appear to be infected. The nature of resistance has not yet been determined.

PI 178383 (highly resistant)

Penetration by the fungus is similar to that in Red Bobs; however, fewer plants are penetrated. Growth of the fungus after

penetration is similar to that in Red Bobs, only much slower. After 50 days, only traces of mycelium can be seen in the leaf tissues and the fungus is situated well above the growing point. It thus appears that PI 178383 is resistant to race D-2 of T. controvera throughout its life cycle.

Tentative Conclusions:

It thus appears that resistance is in part brought about by partial inhibition of initial infection, resulting in fewer plants being penetrated. A further, more significant expression is in the inhibition of growth of the fungus within the wheat tissue, resulting in the slower rate of penetration and the final inability of the fungus to reach the growing point. This latter, of course, leads to full resistance when this exclusion from the growing points is complete in all plants.

TASK 6

THE GEOGRAPHICAL DISTRIBUTION OF TCK SMUT IN MONTANA

Supported by

The Old West Regional Commission
and
The Montana Wheat Research and Marketing Committee

Submitted by

D. E. Mathre, Professor of Plant Pathology

Department of Plant Pathology

Agricultural Experiment Station

Montana State University, Bozeman, Montana

Between May 1973 and January 1975 wheat exports from the United States to the People's Republic of China (PRC) totaled 137,158,000 bushels. However, no wheat has been exported to the PRC from Pacific Northwest Ports since February 1974. The reason for the cessation of shipments from Pacific NW ports was due to contamination of wheat with dwarf bunt (TCK) spores. The PRC believes that this fungus disease is not found in their country so they placed a quarantine against introduction of grain found to be carrying this pathogen.

There are several bunt or smut diseases that attack wheat. Common (or stinking) smut has been known since Biblical days. Dwarf bunt was originally described in the late 1800's but was thought to be a subspecies or variant of common bunt. However, research since then has shown it to be a separate and distinct species of smut that is restricted by environmental factors to geographic areas where snow cover exists for long periods during the winter months. It is known to attack only winter wheat while common bunt can attack both winter and spring wheat.

Most of Montana's wheat that is sold for export is shipped west from the state. An embargo on shipment of all wheat from Pacific NW ports to China effectively eliminates Montana wheat from this market. Previous experience in Montana suggested that most of our wheat is produced in areas that are not conducive to the development of TCK smut and hence should be free of contamination as the grain leaves the farm. This study was undertaken to delineate more specifically the wheat producing areas in Montana that are free from this disease. This information could then perhaps be used to establish areas where the PRC could purchase wheat that was TCK free. Another purpose of our study was to determine possible ways that clean wheat can become contaminated with TCK spores, including grain handling and transportation facilities.

METHODS

Grain samples were collected primarily by field men of the Agricultural Stabilization and Conservation Service (ASCS) of the USDA. Small cloth grain bags that had never been used were provided to these men along with a set of instructions on how samples were to be taken. Samples were collected from commercial grain elevators and grain handling facilities and from grain still located on farms. In a few instances, the author personally collected the samples. Samples were shipped to Bozeman, usually via the US Postal Service. Most of the samples were from the 1974 wheat crop with a few samples from the 1975 crop.

Samples were analyzed for the presence of smut spores by the following technique which is quite similar to the one proposed by the Grain Division, Agricultural Marketing Service, United States Department of Agriculture. This technique is supposedly similar to the one used by the PRC to examine grain for TCK.

Fifty gram samples of grain were removed from the sample bag using a new paper drinking cup. The grain was placed in 250 ml Erlenmeyer flasks that had been washed in a commercial dishwasher that removed any possible TCK spores from the glass surface. One hundred mls of distilled water plus one drop of the surfactant Tween 20 were added to each flask. The flasks plus grain were shaken on a rotary shaker for one minute to release any spores adhering to the grain. Next, the grain-water mixture was strained through new cheesecloth and the filtrate centrifuged at 1000 g's for 2 minutes in new plastic centrifuge tubes. The supernatant was poured off and the precipitate in the bottom of the tubes was removed with an unused disposable Pasteur pipette. This precipitate was placed on a glass microscope slide along with 2 drops of Shear's mounting solution. After a cover slip was added, the slide was heated gently until

bubbles first appeared. When cool, the slides were examined microscopically, first at 100 power. Any suspect TCK spores were examined at 450 or 1000 power to verify their identity. Measurements of spores of the three wheat smuts, Tilletia controversa (TCK), Tilletia caries (Common Smut), and Tilletia foetida (Common Smut) from known sources were made to aid in identification. TCK spores were considered to be those with definite spines and ridges on the spore wall. Spores with similar wall markings but no definite spines or ridges were considered to be T. caries. Spores with smooth walls were considered to be T. foetida. Dr. Jim Hoffman, USDA plant pathologist at Utah State University in Logan, Utah personally examined representative slides of spores we were calling TCK spores and confirmed our diagnosis.

RESULTS

Sensitivity of Analysis.--To determine the level of sensitivity of the grain washing technique, wheat grain known to be free of TCK was contaminated with known amounts of TCK spores as follows:

1) 50 g grain plus one unbroken bunt ball of TCK
2) 50 g grain plus one broken bunt ball of TCK
3) 50 g grain plus 650,000 spores of TCK
4) 50 g grain plus 65,000 spores of TCK
5) 50 g grain

The above samples were then processed as described in the materials section.

The results of this experiment indicated that sample #4 would produce 3-4 TCK spores per 100 power microscope field--a value that we arbitrarily called a "moderate" level of contamination. Samples 1, 2, 3 produced an uncountable (more than 500) number of TCK spores per 100 power microscope field--a value we called "high" contamination. A level we called "trace" was assigned to situations where 1-6 TCK spores were observed in an entire scan

across a microscope slide. Approximately 6500 spores/50 g grain would provide this level of contamination.

The number of TCK spores per bunt ball was determined by actually weighing several balls, crushing them, and suspending the spores in water. Haemocytometer counts were then made on an appropriate dilution of the spore suspension. This indicated that there are approximately 6,700,000 TCK spores per bunt ball. Assuming that there are 50 bunt balls per head, then 335 million spores would be produced by one infected head. Therefore, a "moderate" rating above would be produced when TCK spores from 5 heads would contaminate 25 bushels of grain. A trace level is approximately 1/10 of a moderate level, which means that less than 1 infected head per 25 bushels would produce this level of contamination. If this is translated to percentage of plants infected, and assuming a yield of 25 bushels per acre produced from 500,000 plants or 2 million heads, then a trace level of TCK contamination can be detected when 0.00005% of the plants are infected. If 1% of the plants are infected, this would produce a level of contamination that we called a "heavy" level.

Analysis of Grain Samples.--A total of 302 grain samples were received from 31 different counties in Montana. This included 160 samples from farm storage facilities, 140 from grain elevators and other commercial grain handling facilities and 2 from unknown sources. Forty-one percent of the samples were winter wheat, 22% were spring wheat or durum, and 37% were not designated as to type.

TCK spores were found on a total of 38 samples--22 on winter wheat, 13 on wheat of undesignated type, 2 on spring wheat, and 1 on barley. Of the 38 samples with TCK, 39% were from farm sources and 61% from elevator sources. TCK was previously reported in Montana in the following counties: Flathead, Lake, Missoula, Ravalli, Fergus, Gallatin, and Big Horn. This survey found

TCK on grain from all of the above counties except Flathead from which no samples were received, and in addition from Choteau, Teton, Carbon, and Stillwater counties. Seventy-one percent of the samples from counties previously known to be harboring TCK were found to contain TCK spores while only 26% of the samples from the four "new" counties were contaminated.

The degree or level of contamination with TCK spores was usually very low i.e. trace levels as described above (Table 1). A high level was found on only 4 of the 38 contaminated samples, one each from Big Horn, Fergus, Lake, and Gallatin counties. The levels of contamination in Choteau, Teton, and Carbon counties were all at the trace level which indicates that these areas are only marginally conducive to development of this disease and most likely will never suffer production problems from TCK smut.

Since TCK smut is a disease only of winter wheat, samples of spring wheat or barley found to have TCK spores must have picked the spores up when passing through contaminated grain handling equipment or facilities. That this can happen is illustrated by the finding of TCK on two samples of spring wheat and one of barley. The latter was removed from grain passing through a grain cleaner in an elevator known to be contaminated with TCK. One of the contaminated spring wheat samples also came from an elevator known to be contaminated with TCK.

To obtain more information on TCK contamination of commercial grain handling facilities we visited two elevators in an area previously known to have had TCK infestations. One elevator was very old while the other was of fairly recent construction. Samples were removed from various areas in the elevator and placed in new glassine paper bags for later analysis. In addition, five rail "box cars" were sampled for TCK spores. Dust samples were mounted directly in Shear's solution for observation. The results are shown in Table 2.

It appears that grain handling facilities in areas where some fields produce crops with a high level of TCK infestation, can become contaminated with TCK and can pass this contamination along to clean crops such as spring wheat or barley. In an area where infestations are very light, such as Choteau county, only one contaminated sample came from an elevator, the rest were from farms thus indicating that the grain handling facilities in this area are not highly contaminated.

Overall, in Montana, TCK contamination of wheat remains at a very low level and is restricted to only a few areas. Table 3 summarizes by district all of the samples analyzed.

During the course of this study, it became obvious that small smounts of common stinking smut could be found on many of the samples. Overall, 212 out of the 302 samples contained at least trace amounts of either Tilletia caries or Tilletia foetida spores. Some samples contained only one of the smuts, while others contained both (Table 4). Still others contained TCK plus one or more of the common bunts. This information suggests that even though seed treatment has worked remarkably well in controlling common stinking smut, trace levels exist in over 2/3 of our wheat crop. Therefore, continued treating of wheat seed for control of this disease is a must. Previous studies have shown that the cost of seed treatment is a very inexpensive insurance policy against the potential losses that a grower could incur from this disease. In contrast to TCK, common bunt contamination was observed in samples from all areas of the state.

DISCUSSION AND SUMMARY

The amount and level of TCK smut contamination in Montana has and probably will continue to be quite low. The disease is currently known to occur in levels high enough to cause production losses in only six counties where

total wheat production in 1974 represented less than 10% of Montana's total wheat production. The acreage actually infected is most likely less than 1% of Montana's total wheat acreage. With the availability of Crest and Luke as resistant varieties, this disease should not be a production problem on any Montana farm.

The fact that trace levels of TCK spores were found on some samples is indicative of two things: 1) the sensitivity of the technique whereby infection levels of 1 head per acre can be detected and 2) the fact that certain areas are environmentally conducive to TCK development even if only to a very slight degree.

In regards to providing a source of TCK-free wheat for markets in China, the area of the northern Hi-Line plus the northeastern and southeastern districts are presently free of TCK and most likely will remain so since these areas normally lack the long periods of snow cover required for TCK spores to germinate and infect the winter wheat plant. If 1974 production figures are used for these areas, approximately 66,000,000 bushels of TCK-free wheat (both winter and spring) would have been available. Whether this wheat could have been maintained TCK free as it was transported to the West Coast and then to China is an unanswerable question at this point in time. If China would agree to take wheat with trace levels or less of TCK contamination, then nearly 100% of Montana's wheat crop would qualify.

Table 1. Geographic location of grain samples contaminated with TCK smut in Montana.

County	Total Number of Samples Analyzed	Number of Samples with TCK Smut		
		Trace	Moderate	High
Big Horn	1	0	0	1
Carbon	7	3	0	0
Choteau	37	7	0	0
Fergus	19	11	3	1
Gallatin	9	2	1	1
Lake	3	0	1	1
Missoula	3	3	0	0
Stillwater	4	1	1	0
Teton	1	1	0	0
Total	84	28	6	4

Table 2. Elevator and rail car contamination with TCK smut spores.

Old Elevator	TCK Presence
Wheat residue from ledge in bin	Moderate
Dust near grain scale	Mod-High
Grain and residue from mill floor	Moderate
New Elevator	
Dust near dump pit	Moderate
Dust plus grain near grain collection point	Moderate
Grain near dump pit	Mod-High
Barley going through grain cleaner	Trace
Rail Cars	
1. Grain and dust	Negative
2. Grain and dust	Negative
3. Grain and dust	Negative
4. Grain and dust	Negative
5. Grain and dust	Trace

Table 3. Presence of TCK smut spores on wheat samples in Montana.

District	County	Total Samples Analyzed	# Samples with TCK Smut
NORTHEAST			
	Valley	24	0
	Daniels	11	0
	Sheridan	17	0
	Roosevelt	23	0
	Richland	11	0
	Dawson	5	0
	McCone	44	0
	Garfield	2	0
		137	0
SOUTHEAST			
	Rosebud	15	0
	Prairie	1	0
		16	0
NORTHCENTRAL			
	Glacier	1	0
	Pondera	1	0
	Teton	1	1
	Choteau	37	7
	Toole	13	0
	Liberty	1	0
	Hill	26	0
	Phillips	14	0
		94	8
CENTRAL			
	Fergus	19	15
	Judith Basin	1	0
		20	15
SOUTHCENTRAL			
	Treasure	2	0
	Big Horn	1	1
	Yellowstone	1	0
	Stillwater	4	2
	Carbon	7	3
		15	6
NORTHWEST			
	Lake	3	2
	Missoula	3	3
	Powell	2	0
	Granite	2	0
	Ravalli	1	0
		15	6
SOUTHWEST			
	Gallatin	9	4
	GRAND TOTAL	302	38

Table 4. Contamination of Montana wheat samples with common bunt (*Tilletia caries* and/or *Tilletia foetida*).

Type of Wheat	Smut Spores	Total Wheat Samples Analyzed	Number with Smut
Winter	*Tilletia caries*		54
	Tilletia foetida		24
	T. caries + *T. foetida*		15
		124	93
Spring	*Tilletia caries*		35
	Tilletia foetida		6
	T. caries + *T. foetida*		4
		68	45
Undesignated	*Tilletia caries*		51
	Tilletia foetida		11
	T. caries + *T. foetida*		12
		109	74
	GRAND TOTAL	310	212

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TASK 7

STRENGTHENING THE EXISTING WHEAT BREEDING PROGRAM WITH EMPHASIS ON TCK SMUT RESISTANCE

Supported by

The Old West Regional Commission
and
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Submitted by

C. J. Peterson, Jr., Research Agronomist

R. J. Cook, USDA, ARS

G. L. Rubenthaler, USDA, ARS

Department of Agronomy and Soils

Washington State University, Pullman, Washington

Development of Resistant Varieties:

The objectives of this project will be slow to materialize because they involve the development of new varieties. Crosses between adapted Northwest wheats and known sources of TCK resistance were made in 1975 and 1976. Two hundred and thirty-five crosses were made in 1975 between dwarf bunt resistant lines and wheats adapted to the Pacific Northwest. In 1976, over 200 new crosses were made between TCK resistant lines and adapted cultivars. Many of the 1976 crosses were top crossed with adapted lines or new sources of TCK resistances.

A TCK nursery was established at Rice, Washington in 1974-75 on land known to be infected with TCK. Advanced generation lines (684) were planted in October of 1974. The nursery was inoculated with TCK spores even though the land was known to be infected. Infection was very good and susceptible material had 90-100% of the heads infected. Forty-six lines were identified as being resistant and 22 were given to Dr. Jim Hoffmann at Logan, Utah for further testing. Seed from 9792 F_2 plants, representing 336 crosses, was planted September 30, 1975 at Rice, Washington. Many of the crosses included in this nursery do not carry genes for TCK resistance. They were included in order to better ascertain their agronomic characteristics. An advanced generation nursery of 684 lines was also seeded at Rice. This nursery includes the Western Regional Soft White and Hard Red Nurseries.

Disease notes were taken on this material in August 1976.

Infection was very erratic making it difficult to select resistant lines. Lines showing resistance will be further tested to determine if they were escapes or actually resistant.

Genetic Studies:

A study designed to show the effect the semidwarf trait of wheat has on the incidence of Dwarf Bunt was initiated. The preliminary data suggests that the 2 gene and 1 gene semidwarf levels are more vulnerable to Dwarf Bunt than non-semidwarf wheats. Near-isolines of wheat representing two, one, and no semidwarf genes showed different levels of severity of infection at Logan, Utah, Blue Creek, Utah, and Rice, Washington. The 2 gene levels had 7, 49, and 63% more smutted tillers than their standard height sibs at Logan, Blue Creek, and Rice, respectively.

The 1 gene level had proportionately less infection. This height level had 4, 27, and 70% more smutted tillers than their standard height levels at the three sites. These initial data warrant further study of the relationship between the semidwarf trait and the incidence of Dwarf Bunt in wheat. The implications are that semidwarf wheats may be more vulnerable to loss from this disease than normal height varieties. Plantings of this test were expanded for the 1976-77 trials.

F_1 hybrids of over 200 crosses were grown at Spillman Farm

where they were evaluated for stripe rust and leaf rust reaction as well as rated for overall agronomic appearance. Thirty F_2 populations which had been planted in the fall were also evaluated for foliar disease reaction, presence of favorable plant types, straw characteristics, and segregation pattern for plant height. Of these populations, 15 have been selected for harvest on an individual plant basis. Their progeny will be placed in tests at Rice, Washington in the fall of 1976. These 15 populations carry dwarf bunt resistance from PI 119333, PI 178201, PI 251392, PI 245579 and Luke. The level of dwarf smut infection at Rice this year is extremely erratic which will necessitate retesting many of the 1,000 selections planted there for the 1975-76 crop year.

Effectiveness of Resistance in Combination with Fungicides Under Various Environmental Conditions:

In the trial for integrated control of dwarf smut using a combination of resistant varieties, early seeding, and fungicides, virtually no dwarf smut developed in any of the plots. Even the most susceptible variety used, Wanser, seeded late and with no fungicide, developed less than 5% smut, and in many plots, less than 1% smut. Although counts were made of smut incidence in each plot, the numbers are so low as to be meaningless. The soil was frozen during most of the winter, due to low temperatures prior to snowfall. This circumstance is believed to be the cause for the

very unusual and disappointingly low levels of smut.

The trial is again underway with the early seeding completed August 31 and the late seeding, October 5, 1976. Several varieties with varying degrees of smut resistance were included. Half of each plot was inoculated with additional smut spores on October 28 in case the natural inoculum is adequate for a good test. TBZ (Thiabendizole) was applied as a spray on the leaves and soil surface of one complete set of plots on October 28. For every plot sprayed, two are not sprayed to test the influence of early seeding on incidence of smut in the different wheats. Another complete experiment was made with wheat seeded in ridges instead of furrows since furrows are known to concentrate the smut inoculum in the row in ideal proximity to the plant. Possibly ridges will reduce smut. Results of these experiments will not be available until July, 1977.

Quality:

Milling and flour quality tests were conducted on the advanced generation material that was seeded at Rice, Washington (1975-76). The following tests were run: 1) milling, 2) protein analysis, and 3) physical dough properties. This information will be used to help the plant breeder determine which lines will be saved for futher testing.